The Structure, Biosynthesis and Functions of Surface Glycoconjugates in *Trichomonas vaginalis*

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M.Res. Dipl.-Biochem.

Submitted in fulfilment of the requirements for the Degree of PhD

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December 2010

Abstract

Trichomonas vaginalis causes trichomoniasis, a common sexual transmitted disease. This disease has been considered as a major public health problem, as it is associated with increased HIV transmission, adverse birth outcome and prostate cancer development. Recently, attention has been drawn to the characterization of the *T. vaginalis* surface molecules important for disease progression. Glycosylphosphatidylinositol (GPI) molecules are considered ubiquitous in eukaryotic organisms and are particularly abundant on the surface of parasitic protozoa, where they play essential roles in parasite survival, infectivity and pathogenesis. *T. vaginalis,* however, has a variety of surface virulence factors with single transmembrane domains, one exception being the major surface lipophosphoglycan-like molecule TvLPG.

TvLPG is an important virulence factor involved in cytotoxicity and adhesion to the host's cells. Although very little is known about the primary structure of TvLPG, it is well established that this molecule is a lipopolysaccharide rich in glycans and attached to the parasite membrane via a lipid anchor. The biosynthesis and transfer of GPI molecules to proteins is a posttranslational modification that involves a minimum of 15 gene products. Interestingly, none of the conserved genes involved in the biosynthesis and transfer of GPI molecules were found to be present in the genome of *T. vaginalis,* indicating that TvLPG has an unusual lipid anchor. Here, using a combination of cell-free systems and metabolic incorporation of radioactive sugar precursors, it was shown that *T. vaginalis* is indeed unable to make "classical" (*i.e.* with a glycan core made of mannose and glucosamine residues) GPI molecules. Using a combination of mass spectrometry and chemical treatments, together with the *in vivo* incorporation of radioactive precursors, it was determined that: 1) TvLPG does not contain peptides or amino acids, further corroborating its identity as a lipopolysaccharide; 2) rhamnose, galactose and *N*-acetylglucosamine are the main constituents of this glycoconjugate; and 3) TvLPG is susceptible to the action of phosphatidylinositol-phospholipase C (PI-PLC)

and serum phospholipase D (PLD), but resistant to mild alkaline treatment, suggesting inositolphosphoceramide (IPC) as its main phospholipid anchor.

Furthermore, the compositional analysis corroborated rhamnose as the major carbohydrate component of TvLPG making the rhamnose biosynthetic pathway a possible drug target for the treatment of trichomoniasis. It was further shown that classical and novel approaches can be combined to identify and validate potential drug target genes essential for TvLPG biosynthesis starting with the rhamnose biosynthetic pathway. Digital transcriptomics analysis combined with sugar nucleotide pool analysis and bioinformatics was carried out to investigate genes involved in rhamnose biosynthesis and additional genes involved in sugar nucleotide, inositol and sphingolipid biosynthesis and sugar transfer. The analysis revealed that all single copy genes and at least one copy of a multiple copy gene family were expressed. During the analysis, single copy genes important for TvLPG biosynthesis were identified and were mostly derived from genes involved in sugar nucleotide biosynthesis rather than from genes involved in inositol and sphingolipd biosynthesis and sugar transfer. The list included five genes of the sugar nucleotide metabolic pathway, three genes of the inositol metabolic pathway, two genes of the sphingolipid metabolic pathway and one glycosyltransferase gene. However, only one gene (UDP-*N*-acetylglucosamine pyrophosphorylase guanylyltransferase) was considered as additional targets, as they were single copy and important for biosynthesis of UDP-GlcNAc, important building blocks of TvLPG. A disruption of the remaining genes would not have disrupted the biosynthesis of TvLPG precursors, as alternative routes were available which could be taken to synthesis a TvLPG precursor. Further, at the genetic level, several novel strategies were attempted to generate gene-deficient parasites, including a lectin selection approach, a positive/negative selection approach and a destabilisation domain approach, as the classical approach using targeted gene replacement with a double cross-over event did not yield gene-deficient parasites with correct integration. Two genes involved in rhamnose biosynthesis, *TvrmlC-1* (Tvag_313960) and *TvrmlD* (encoding for

UDP-6-deoxy-*D*-xylo-4-hexulose 3,5-epimerase and UDP-6-deoxy-*L*-lyxo-4-hexulose reductase, respectively) were chosen as targets. Low transfection efficiency and poor luck prevented the generation of *TvrmlC-1* and *TvrmlD* knockout parasites. As no additional selectable marker apart from the neomycin phosphotransferase gene was available, several additional selectable markers were tested. Although puromycin *N*-acetyltransferase did not work to confer resistance to puromycin in transgenic *T. vaginalis* parasites, streptothricin acetyltransferase and blasticidin S-deaminase still need to be tested, as both antibiotics were able to kill wild-type parasites. On the protein level, out of the four putative proteins involved in rhamnose biosynthetis in *T. vagingalis*, TvrmlB-D were expressed as recombinant proteins to establish a linked assay for TvrmlD activity tests. Preliminary results were obtained for TvrmlB characterization, supporting the idea that the recombinant protein of TvrmlB is active in a modified bacterial rmlB activity assay, while the biochemical characterization of TvrmlC and TvrmlD is pending.